

Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing
Chicken Embryo-Report of the in-house investigation of **Ethyl Formate** in the
developing chicken embryo 12/29/77

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MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : GRAS Review Branch, HFF-335

DATE: December 29, 1977

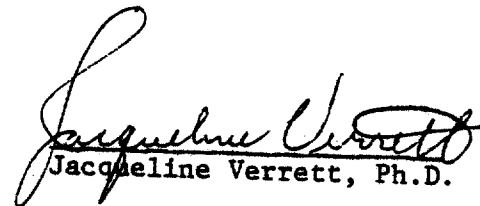
Through: HFF-150 _____

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FROM : Supervisory Chemist
Whole Animal Toxicology (HFF-155)

SUBJECT: Investigation of the Toxic and Teratogenic Effects
of GRAS Substances to the Developing Chicken Embryo

Attached is the report of the inhouse investigation of Ethyl
Formate in the developing chicken embryo.


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Investigations of the Toxic and Teratogenic Effects of
GRAS Substances to the Developing Chicken
Embryo: Ethyl Formate

Protocol:

Ethyl Formate (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in ethanol as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some to hatch.

Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of test.

Columns 1 and 2 gave the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Ethyl formate showed very little toxicity when administered up to 500 mg/kg (25 mg/egg). Air cell treatment at 0 hours resulted in a calculated LD₅₀ of > 4000 mg/kg (>200 mg/egg). For the other three test conditions no LD₅₀ could be calculated.

Scattered abnormalities were observed under all conditions of test, but serious abnormalities were in no instance significantly higher than or different from those observed in the background. Ethyl formate displayed no teratogenicity under the test conditions employed.

1. Ethyl Formate, Source Unknown
2. McLaughlin, J., Jr., Marliac, J. P., Verrett, N. Jacqueline, Mutchler, Mary K., and Fitzhugh, O. G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770
3. Verrett, N. J., Marliac, J. P., and McLaughlin, J., Jr., (1964) JAOC 47, 1002-1006
4. Finney, D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I

Ethyl Formate Air Cell @ 0 Hours

Table 1

mg/egg	Dose mg/kg	Number of Eggs	** Percent Mortality	Percent Abnormal	
				Total	Structural
25.00	500.00	105	46.66*	6.66	4.76
12.50	250.00	105	37.14	3.80	2.85
5.00	100.00	105	28.57	5.71	5.71
2.50	50.00	105	22.85	1.90	0.95
1.250	25.00	105	23.80	0.95	0.95
Ethanol		105	32.38	0.95	0.95
Controls		407	7.61	0.98	0.98

*Significantly different from solvent $p \leq 0.05$

**LD₅₀ > 4000 mg/kg (> 200 mg/egg)

Ethyl Formate Air Cell @ 96 Hours

Table 2

mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal	
				Total	Structural
1.00	20.00	115	46.95	2.60	0.86
0.50	10.00	115	48.69	4.34	1.73
0.20	4.00	114	43.85	4.38	0.87
0.10	2.00	115	54.78*	6.95	2.60
0.050	1.00	115	43.47	8.69	3.47
Ethanol		115	36.52	3.47	2.60
Controls		407	7.61	0.98	0.98

*Significantly different from solvent $p \leq 0.05$

**Slope not significantly different from zero $p = 0.05$

Ethyl Formate Yolk @ 0 Hours

Table 3

mg/egg	Dose mg/kg	Number of Eggs	** Percent Mortality	Percent Abnormal	
				Total	Structural
25.00	500.00	105	53.33*	1.90	0.95
12.50	250.00	105	39.04	4.76	1.90
5.00	100.00	105	35.23	0.95	0.95
2.50	50.00	105	42.85*	2.85	0.00
1.250	25.00	105	41.90*	2.85	1.90
Ethanol		105	26.66	0.00	0.00
Controls		407	7.61	0.98	0.98

*Significantly different from solvent $p \leq 0.05$

**Slope not significantly different from zero $p = 0.05$

Ethyl Formate
Yolk at 96 Hours

Table 4

mg/egg	Dose mg/kg	Number of Eggs	** Percent Mortality	Percent Abnormal	
				Total	Structural
1.00	20.00	115	45.21	0.86	0.00
0.50	10.00	115	49.56*	6.95	5.21
0.20	4.00	115	37.39	1.73	1.73
0.10	2.00	115	34.78	5.21	4.34
0.050	1.00	115	40.86*	4.34	1.73
Ethanol		115	26.08	3.47	1.73
Controls		407	7.61	0.98	0.98

*Significantly different from solvent $p \leq 0.05$

**Slope is negative